REMARKS

Claims 58-103 are currently pending in this application. Claims 58-92 have been rejected under 35 U.S.C. § 112, first paragraph, for lack of written description. Claims 60-63 and 72-90 are rejected under 35 U.S.C. § 112, second paragraph, for lack of clarity. Finally, claims 60-92 are rejected for obviousness-type double patenting over claims 1-25 of U.S. Patent No. 6,251,590 in view of Chee et al. (U.S. Patent No. 5,837,832). Each of the rejections raised by the Examiner is discussed below. Applicants note that the Examiner has not indicated the current status of claim 93 and request that the Examiner provide this information in the next office communication.

Support for the Amendments

Support for the amendment to claims 58 and 59 is found, e.g., on page 2, lines 8-10. Support for the amendment to claims 72, 73, 75, 76, 80, 81, 83, and 84 is found in former claims 72, 73, 75, 76, 80, 81, 83, and 84, respectively. Support for new claims 93-103 is found in the specification on, e.g., page 53, lines 4-17. No new matter is added by the amendment.

Obviousness-Type Double-Patenting Rejection

Claims 60-92 have been rejected under the judicially-created doctrine of obviousness-type double patenting as being unpatentable over claims 1-25 of US Patent No. 6,251,590, in view of Chee et al. (U.S. Patent No. 5,837,832). If the pending claims are found to be otherwise allowable except for this ground of rejection, Applicants will submit an appropriate terminal disclaimer. In this event, Applicants request that the Examiner telephone the undersigned who will then provide the terminal disclaimer.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 58-92 stand rejected under 35 U.S.C. § 112, first paragraph, for lack of an adequate written description. The Examiner states that although the specification discloses methods of obtaining nucleic acids that correspond to spliced regions, the mere recitation of exon, intron, or junctions of spliced variants does not provide the requisite level of description to

support claims to a product obtained using nucleic acids comprising these regions. Applicants respectfully traverse the rejection.

The M.P.E.P. § 2164.05(a) states:

> The specification need not disclose what is well-known to those skilled in the art and preferably omits that which is well-known to those skilled and already available to the public. *In re Buchner*, 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991); *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384, 231 USPQ 81, 94 (Fed. Cir. 1986), *cert. denied*, 480 U.S. 947 (1987); and *Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co.*, 730 F.2d 1452, 1463, 221 USPQ 481, 489 (Fed. Cir. 1984).

Applicants' present claims are directed to products comprising a support material and a plurality of different nucleic acid molecules that are complementary to and specific for an exon or intron of a gene, or an exon-exon or exon-intron junction region of a gene or RNA. The Examiner argues that Applicants' specification fails to adequately describe the common attributes of the genus of nucleic acid molecules for use in the claimed products. Applicants contend that it is not necessary to describe the genus of nucleic acid molecules in any more detail than that already provided in the specification, and further, that it would be more than is necessary because this information is already well known to the skilled artisan (see, e.g., M.P.E.P. § 2164.05(a), *supra*). Our reasons follow.

Trans-splicing was Well Known to the Skilled Artisan

It was well appreciated prior to Applicants' invention that during the process of transcription of a gene, large regions of intervening sequences within an mRNA transcript derived from the gene, termed introns, are removed, leaving only the sequences required for protein expression (i.e., exons). Exons and introns, as well as the junction regions that span the exon-exon or exon-intron sequences of genes, were well-known to, or easily identified by, the skilled artisan prior to Applicants filing date based on this knowledge of the process of gene transcription. Evidence of this can be found by performing a search using, e.g., PubMed (www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed). A search of this database using the keywords "alternative splicing," "differential splicing," and "trans-splicing," and limiting the

search to articles published prior to March 24, 1998, the earliest priority date for the present application, yields a search result of 6,519 references; the earliest published in 1979. Many of these references identify not only the presence of differentially-spliced gene products (i.e., the "normal transcript" and the "differentially-spliced transcript(s)"), but also the specific region of variability between the various transcripts (see below). Therefore, exons, introns, and junction regions of differentially-spliced genes, and the nucleic acid molecules corresponding to these regions, were known in the art. Accordingly, the metes and bounds of these terms would have been clearly understood by the skilled artisan reading Applicants' specification at the time the application was filed.

Furthermore, the instant invention discloses a novel concept of genetic analysis and functional genomics, and provides novel products for performing such analyses. While the occurrence of splicing events during gene expression was known and specific splicing variants of particular genes had been identified, no prior art of record disclosed or suggested in any way a method based on the monitoring of differential alternative exon or intron splicings, the manufacture of products comprising a plurality of nucleic acid sequences specific for splicing events for monitoring the occurrence of differential alternative exon or intron splicings in a sample, or the advantage of monitoring, in a sample, genetic variability due to differential splicing events, as presently disclosed and claimed.

The Requirement that Applicants Call Out Each and Every Nucleic Acid Molecule Corresponding to an Exon, Intron, or Junction Region of a Gene is Unreasonable

The presently claimed products comprise a plurality of different nucleic acid molecules attached to a support material. The nucleic acid molecules are complementary to and specific for an exon or intron of a gene, or an exon-exon or exon-intron junction region of a gene or RNA. The Examiner states that the product claims recite a broad and widely varying genus and there is no description of all the possible varying spliced exons, introns, and junctions, or any of the common attributes thereof (Office Action, p. 4). It would be unreasonable for Applicants to describe each and every one of the thousands of such nucleic acid molecules corresponding to exons, introns, or junction regions of a gene or RNA that could be utilized in the presently

claimed products because nucleic acid molecules for use in the presently claimed products are easily recognized by the skilled artisan working in the field of molecular and cellular biology based on a) their previous disclosure in published research articles, b) their availability from DNA libraries or from computerized data base systems that compile information about exons, introns, and junction regions of differentially-spliced genes, or c) their identification using the methods provided in the instant specification (see below). Further, the M.P.E.P. § 2163 states:

> The written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice..., or by disclosure of relevant, identifying characteristics, i.e., structure and or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show applicant was in possession of the claimed genus... See *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406.

Applicants' have clearly satisfied this standard, as the specification describes a representative number of species of nucleic acid molecules that one skilled in the art could use to make the presently claimed products, as well as relevant, identifying characteristics (i.e., the nucleic acid molecules corresponding to exons, introns, or junctions regions of differentially-spliced genes). Furthermore, the examples disclosed in the specification, coupled with the knowledge and information available to one of skill in the art, provide considerable teachings sufficient to show Applicants were in possession of the presently claimed products.

The Specification Provides Examples of Previously Described Nucleic Acid Molecules

One representative example of a nucleic acid molecule that can be used in any of the presently claimed products is exon 2 of the p130 protein of the retinoblastoma family. The specification discloses that p130 is mutated at a consensus splicing site in cells of patients with small cell lung carcinoma (see page 29, lines 10-13). This mutation results in the removal of exon 2 and loss of synthesis of the protein due to a premature stop codon. The complete sequence of the p130 protein, including exons, introns, and junction regions was disclosed prior to Applicants' priority date by Baldi et al. (Proc. Natl. Acad. Sci. U.S.A. 93:4629-4632, 1996; a

copy of which is provided herewith). Based on the specification and the knowledge of the p130 genomic sequence, one skilled in the art could easily provide one or more sequences from this gene as the nucleic acid molecules for use in the presently claimed products.

A second representative example of a nucleic acid molecule that can be used in the presently claimed products is the junction region comprising the mutated donor splice site of the p16INK4a gene (see, e.g., Shapiro et al., Cancer Res. 55:6200-6209, 1995; a copy of which is provided herewith; see also GenBank Accession No. L27211). The specification teaches that p16INK4a is an inhibitor of cyclin-dependent kinases cdk4 and cdk6 and is mutated in certain non small cell lung carcinomas. Furthermore, "[t]his mutation results in the production of a truncated protein with a short half-life, leading to the accumulation of the inactive phosphorylated forms of [retinoblastoma]" (see page 29, lines 15-19). Accordingly, exons, introns, or junction regions of this gene can also serve as the nucleic acid molecules for use in the presently claimed products.

The specification also describes differentially-spliced variants of the Wilt's tumor suppressor gene (WT1), the neurofibrin NF1 gene, the HDM2 gene, and the p53 gene, the sequences of which were all available prior to Applicants' filing date, and which can be used in the presently claimed products (see, e.g., page 29, lines 19-32).

The Specification Describes Nucleic Acid Molecules Present in DNA Libraries or Identified in Computerized Data Base Systems

The specification discloses that nucleic acid molecules present in a cDNA library representative of a given physiological state of a biological sample (e.g., a disease state, such as cancer) can be used as a source of the nucleic acid molecules of the presently claimed products. The specification teaches that these libraries, which consist of cDNA inserted into plasmid or phage vectors, can be deposited on support materials to produce the presently claimed products (see, e.g., page 19, line 13, through page 23, line 23). These libraries can be easily produced or obtained by one skilled in the art and, following the teachings contained in the present specification, nucleic acid molecules derived therefrom can be used in the presently claimed products.

The specification also teaches:

> Alternative intron or exon libraries can also be in the form of computerized data base systems compiled by systematically analysing databases in which information about genomes of individual organisms, tissues, or cell cultures is recorded. In such a case, the data obtained by elaboration of such virtual databases may be used to generate oligonucleotide primers that will serve in testing two pathophysiological conditions in parallel. (See page 23, lines 24-29.)

As evidence that this source of nucleic acid molecules for use in the presently claimed products is readily available to the skilled artisan, Applicants direct the Examiner to the following internet sites which provide access to databases of differentially-spliced genes, including specific exons, introns, and junction regions: http://hazelton.lbl.gov/~teplitski/alt/; http://www.ebi.ac.uk/asd/; and http://www.bioinformatics.ucla.edu/~splice/HASDB/. Following the teachings contained in the present application, one skilled in the art can use such databases to generate the presently claimed products.

The Specification Describes Nucleic Acid Molecules Identified Using Methods Provided by Applicants

The specification also describes nucleic acid molecules for use in the presently claimed products that are identified using methods provided in Applicants' specification. These methods can be used to identify exons, introns, or junction regions of differentially-spliced genes.

The first method described in the specification uses DNA/RNA heteroduplexes to identify nucleic acid molecules corresponding to differentially-spliced regions (see, e.g., page 14, lines 5, through page 15, line 8). This method generates RNA molecules corresponding to regions of unpaired RNA (due to differential splicing events). It is unnecessary to specifically identify the sequence of the RNA molecule generated by the method; the RNA needs only to be added to a substrate to produce the presently claimed products. The written description standard the *Examiner* has applied would require Applicants to provide more detail in the specification than is required to fully practice the invention.

Another approach described in the specification utilizes random or semi-random primers

and a reverse transcription reaction (see page 15, line 9, through page 17, line 10). This method entails formation of double stranded RNA, which is subsequently digested by, e.g., RNase H, followed by reverse transcription of the remaining RNA molecules. This technique can be used to produce long RNA molecules, which again do not need to be specifically sequenced or identified prior to use, but can simply be added to a substrate to produce any one of the presently claimed products. Variations on these methods for isolating nucleic acid molecules are described in the specification on, e.g., page 17, line 11, through page 19, line 11. As above, the standard the Examiner has applied would require Applicants to provide more detail in the specification than is required to fully practice the invention.

Finally, the specification describes antisense nucleic acid molecules that are generated from RNA fragments identified according to methods provided in the specification (see, e.g., page 24, lines 3-32; methods described on pages 42-53). The antisense nucleic acid molecules, once generated, can be added to a substrate to produce any one of the presently claimed products without the need for sequencing of the nucleic acid molecules.

Based on the above comments, Applicants submit that, contrary to the Examiner's statement, the recitation of exon, intron, or junction regions of a differentially-spliced gene does provide the requisite level of description to support claims to a product obtained using nucleic acids molecules comprising these regions. The specification clearly provides a representative number of species sufficient to identify the genus of nucleic acid molecules for use in the presently claimed products.

The Prior Art also Discloses Exons, Introns, and Junction Regions of Differentially-Spliced Genes

In further support of Applicants' contention that the prior art discloses differentially-spliced genes, including the specific nucleic acid molecules that are either retained or spliced out of the "normal transcript," which can be used in Applicants' presently claimed products, Applicants direct the Examiner to three exemplary references, Le Fur et al. (P.N.A.S. 94:5332-5337, 1997; provided herewith), Raben et al. (Human Molecular Genetics 5:995-1000, 1996; provided herewith), and Famiglietti et al. (J. Cell Biol. 138:1425-1435, 1997; provided

herewith), all of which were published prior to Applicants' priority date. These references further demonstrate that skilled artisans were capable of identifying nucleic acid molecules corresponding to exons, introns, and junction regions of differentially-spliced genes and, using the teaching of the present specification, these artisans could have applied these nucleic acid molecules to substrates to make the presently claimed products.

Applicants first direct the Examiner to Le Fur et al., which describes the identification of alternative splice variants of tyrosinase in murine melanomas. Melanomas tend to become less pigmented during the course of malignant progression, and one of the genes responsible for normal pigmentation is the tyrosinase gene (see, e.g., the abstract). Le Fur et al. states that loss of normal pigmentation is associated with an increase in alternatively-spliced tyrosinase gene transcripts. Le Fur et al. states:

> Deletion of exon 3 (Δ3) was the most frequent type of alternative splicing...Next in abundance was Δ1e, one of several splice variants resulting from a deletion within exon 1...
>
> In comparison with normal skin, virtually all samples from the zonal primary melanomas showed a specific increase of the Δ1b and Δ1d alternative splice variants...The Δ1b and Δ1d mRNAs represent deletions within exon 1 that are due, respectively, to usage of a splice donor site at nucleotides 291 and 369, in conjunction with shared usage of the splice acceptor site at the 5' end of exon 2 (nucleotide 881 in the cDNA sequence). (Page 5335, col. 1.)

Therefore, exon 1 or 2 of the tyrosinase gene, both of which have been identified as a region of variability due to differential splicing in melanomas, could be selected as a nucleic acid molecule for use in the presently claimed products.

Raben et al. discloses that several patients with glycogenosis type II, a recessively inherited disorder caused by mutations in the acid maltase (GAA) gene, express a differentially-spliced variant which results in the deletion of exon 2 of the GAA gene. Expression of the differentially-spliced gene product results in a defect in the hydrolysis of α-1,4 and α-1,6 linkages in glycogen, maltose, and isomaltose, eventually causing death in adults due to respiratory failure. Therefore, exon 2 of the GAA gene, which has been identified as a region of variability due to differential splicing in cases of glycogenosis type II, could be selected as a nucleic acid

molecule for use in the presently claimed products.

Finally, Famiglietti et al. describes the differential splicing of exon 14 of platelet/endothelial cell adhesion molecule (PECAM). The presence of exon 14 results in a PECAM polypeptide that mediates heterophilic aggregation, while the absence of exon 14 produces a PECAM polypeptide that exhibits homophilic aggregation (see, e.g., page 1426, col. 1). Therefore, exon 14 of PECAM, which has been identified as a region of variability due to differential splicing, could be selected as a nucleic acid molecule for use in the presently claimed products for, e.g., identifying compounds that modulate the transition between homophilic and heterophilic aggregation due to the differential splicing of PECAM.

These references show that the skilled artisan was not only aware of differential splicing events in connection with genes, but could identify the exon or intron, or exon-exon or exon-intron junctions involved in the differential splicing. Furthermore, as is taught by Applicants' specification, the nucleic acid molecules which correspond to regions of differential splicing, and which are identified in references that disclose differentially-spliced genes and the regions of variability present in the transcripts derived therefrom, can be used, in addition to the nucleic acid molecules identified using the methods described in the present specification, in Applicants' presently claimed products (see, e.g., page 22, line 25, through page 23, line 29, with particular attention to page 23, lines 3-5, and pages 42-61, of the specification). Therefore, based on the above remarks, and in light of the three references discussed above, Applicants' specification clearly conveys to the skilled artisan that the inventors were in possession of the claimed invention at the time of filing, and provides considerable guidance that would place the skilled artisan in a position to carry out the full breadth of the claimed invention, e.g., to identify splicing events, to identify the polynucleotides specific for such splicing events, and to arrange such polynucleotides on a support, as is illustrated in the working examples. It is therefore submitted that claims 58-62 comply with the requirements of 35 U.S.C. § 112, first paragraph, and withdrawal of this rejection is respectfully requested. Applicants further note that the above comments apply equally to newly presented claims 94-103, related to methods of making such products.

While the above comments describe the knowledge and skill of one in the relevant art at

the time the application was filed with respect to differential splicing events and the recognition and identification of differentially-spliced exon, intron, and junction regions of genes, Applicants reiterate that the instant invention alone discloses a novel concept of genetic analysis and functional genomics that utilizes this knowledge of differential splicing events and provides novel products for performing such analyses. No prior art of record describes the use of exon, intron, and junction regions of genes in methods for monitoring differential splicing events, for the manufacture of products comprising a plurality of nucleic acid sequences specific for splicing events for monitoring the occurrence of differential alternative exons and introns splicings in a sample, or the advantage of monitoring, in a sample, genetic variability due to differential splicing events, as is presently disclosed and claimed.

Claim rejection under 35 U.S.C. § 112, second paragraph

Claims 60-63 and 72-90 have been rejected under 35 U.S.C. § 112, second paragraph, for lack of clarity. The Examiner states that "[i]t is unclear as to what properties the nucleic acids are to possess other than hybridization to exon or intron or junction" (Office Action, p. 6). Applicants respectfully disagree and provide the following clarifying remarks.

Claims 58, 59, 72, 73, 80, 81, 88, 89, and 90, and claims dependent therefrom, in addition to reciting that the nucleic acid molecules hybridize to an exon, intron, or junction region of a gene, recite that the nucleic acid molecules correspond to a region of a gene that is either retained in an mRNA transcript or removed from an mRNA transcript by splicing (i.e., a variable region). Therefore, it would be understood by one skilled in the art that the nucleic acid molecules recited in the present claims are chosen not only for their complementarity to an exon, intron, or junction region of a gene, but also because they correspond to a region within an RNA transcript that varies due to a differential splicing event.

As is discussed above, following the teachings contained in the present specification, one skilled in the art can identify nucleic acid molecules that correspond to a differentially-spliced region of a gene. Therefore, in addition to the recitation that the nucleic acid molecules are cDNA or single-stranded oligonucleotides, as was discussed in the previous reply to Office Action filed on December 30, 2002, Applicants submit that the recitation in the present claims

that the nucleic acid molecules correspond to introns or exons, or exon-exon or exon-intron junction regions that are retained or spliced in a cell, defines a further property of the nucleic acid molecules, the breadth and scope of which is fully and clearly conveyed within the present claims.

The Examiner also states that "[i]t is unclear as to limitation the language treatment by reference toxic compound would impart on a product claim" (Office Action, p. 6). Claims 72, 73, 75, 76, 80, 81, 83, and 84 have been amended to delete this phrase. Accordingly, Applicants submit that the claims, as presently amended, particularly point out and distinctly claim the subject matter regarded as the invention, and respectfully request that the rejection of claims 60-63 and 72-90 under 35 U.S.C. §112, second paragraph, be withdrawn

CONCLUSION

In light of the foregoing amendment and remarks, Applicants submit that the claims are novel and inventive over the prior art and respectfully request favorable reconsideration of the present application. In particular, it is believed that the claims are now in condition for allowance, and a notification to that effect is earnestly solicited.

Enclosed is a Petition to extend the period for replying to the Office Action for two months, to and including September 5, 2003. Also enclosed is a check in the amount of $205.00 for the payment of the petition fee as required by 37 C.F.R. 1.17(a)(1). If there are any additional charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: September 5, 2003

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